CHEPTER 5

CONCLUSION

Data about the DNA content in zinnia has not been published so far. Compared to other species of the family Asteraceae, *Z. angustifolia* (2C: 1 pg.), *Z. haagena* (2C: 1.7 pg.) and *Z. elegans* (2C: 2.6 pg.) have a rather low DNA content. There are significant differences in the DNA content between the three analyzed zinnia species. The flow cytometric estimation of the DNA content can be used as a tool to distinguish between the species at early developmental stages, when other, morphological markers are not yet available. However, the discrimination of *Z. elegans* cultivars by flow cytometry proved unfeasible. When mixed samples of two different cultivars of *Z. elegans* were analyzed, only a single peak could be detected. This clearly demonstrates that the DNA contents of these cultivars are identical.

By analyzing the DNA content of the interspecific hybrid 'Profusion' (*Z. angustifolia* x *Z. elegans*) and of its parents we could show that 'Profusion' is a tetraploid hybrid. By analyzing the morphology of guard cells of the three zinnia species, we could demonstrate that there is a positive and significant correlation between the DNA content and the average guard cell size. The same positive correlation has also been observed between the DNA content and the number of chloroplasts per guard cell.

Those findings are in accordance with the reports of other species in angiosperms (Tsuji *et al*., 1979; Qin and Rotino, 1995). Consequently, these morphometrical characteristics can be used to discriminate species in the genus *Zinnia*

if flow cytometry is not available. However, flow cytometry is the preferable method because it is able to detect chimeras in contrast to the morphometrical methods which are restricted to the epidermal cell layer.

The chromosome numbers of *Z. angustifolia* (2n = 22) *Z. haageana* (2n = 24) and *Z. elegans* (2n = 24) have been previously investigated. Our own observations support those previous reports. However, the *Z. elegans* cv. 'Jungle' has a chromosome number of 48, and DNA content was double values of diploid zinnia thus it is a tetraploid cultivar. The DNA content of *Z. haageana* and *Z. elegans* is significantly different while the number of chromosomes is identical. It might be caused by the evolutionary accumulation of repetitive DNA sequences and/or retrotransposons.

Intraspecific variation in the *Z. elegans* cultivars could not be detected by flow cytometry, by counting the number of chloroplasts in guard cells, or by analyzing the size of guard cells. Therefore, we tried to establish whether RAPD profile is a suitable method to investigate intraspecific variation. Among the 10 primers screened, five primers yielded the best products for analysis. Intraspecific variation was investigated among twenty samples of cv. 'Persian carpet' and *Z. elegans* cv. 'Dreamland', each. The *Z. elegans* and *Z. haageana* studied here showed moderate levels of interspecific genetic variability as shown by a genetic identity of 0.76. Interestingly, the intraspecific genetic identity is considerably lower for *Z. haageana* cv. 'Persian carpet' (47.99%) than in *Z. elegans* cv. 'Dreamland' (81.49%). This is in accordance with the morphological characters (e.g. flower color and color ratios), where *Z. haageana* cv. 'Persian carpet' also shows a higher variation than *Z. elegans* cv. 'Dreamland'.

The results of our initial RAPD analyses indicate the potential of this technique for the discrimination of closely related varieties. We found molecular markers partially coupled to genetic background of certain varieties with the use of just 5 randomly picked primers. A more thoroughly analysis of this approach will likely allow for accurate assessment of zinnia accessions using molecular makers.

In general, our results demonstrate that flow cytometry as well as guard cell size, number of chromosome accurately distinguishes the ploidy levels of diploid, allopolyploid and tetraploid plants in the genus *Zinnia*. The combination of RAPD analyses and flow cytometry techniques can provide an efficient screening tool of cultivars, assessing the genetic origin of aberrant plants, and quantifying the contribution of parental genomes.

In vitro culture of three species of zinnia was investigated. It was found that the appropriate medium formula was KS that had been used to culture mesophyll cells of zinnia previously. KS was supplemented with kinetin and IBA because this type of growth regulator induced more axillary shoots, buds and rooting than other growth regulators. In addition, 2 mg/l $AgNO_3$ promoted axillary shoots. An efficient plant regeneration system from shoot tips has been developed in the three zinnia cultivars. $AgNO_3$ influences shoot organogenesis and this mode of action is cultivar dependent.

In callus induction and organogenesis from callus, the morphological characters of callus depended on the types and concentrations of growth regulators. When *in vitro* shoots were cultured for a long time, the shoots matured and flower buds developed. But the flowers did not bloom normally.